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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PREVENTION, PESTICIDES, AND TOXIC SUBSTANCES
WASHINGTON, D.C. 20460

October 22, 2002

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

Subject: **Methyl Bromide** - Review of Subchronic (6-week) Inhalation Toxicity Study in Dogs

P.C. CODE: 053201
DP Barcode: D284598
MRID NO.: 45722801

TXR NO.: 0050984
SUBMISSION CODE: S619315

To: Joseph Nevola
Special Review Branch
Special Review and Reregistration Division (7508C)

From: Paul Chin, Ph.D. *Paul Chin*
Reregistration Branch I
Health Effects Division (7509C)

Through: Whang Phang, Ph.D. *Whang Phang*
Senior Scientist
Reregistration Branch I
Health Effects Division (7509C)

Alliance of the Methyl Bromide Industry submitted Subchronic (6-week) Inhalation Toxicity Study in Dogs. This study was reviewed by the contractor, Oak Ridge National Laboratory. The DER was appropriately modified to reflect the current policy and guidelines of the Agency. The DER for this study is attached to this memorandum. The citation and conclusion are presented below:

CITATION: Schaefer, G. J., (2002). A 6-week inhalation toxicity study of methyl bromide in dogs. WIL Research Laboratories, Inc., Ashland, OH. Laboratory Report No. WIL-440001. May 16, 2002. MRID 45722801. Unpublished.

EXECUTIVE SUMMARY: In a six-week nonguideline inhalation toxicity study (MRID

45722801), four groups of beagle dogs consisting of 4 males and 4 females/group were administered methyl bromide (Lot No: 1010PK15A; purity: 100% a.i.) by whole body exposure at concentrations of 0, 5.3, 10, and 20 ppm. The exposures were for seven hours/day, five days/week for six weeks (total of 30 exposures). There were no compound related effects on mortality, clinical signs, body weight, food consumption, spleen weights, or gross or histological pathology. Functional observational battery and locomotor activity tests showed no abnormalities relative to controls with one exception. One male and one female at the 20 ppm dose and one male at the 10 ppm dose demonstrated an absence of proprioceptive placing, although no evidence of weakness in motor strength or other signs of neurotoxicity was found.

The LOAEL for methyl bromide was 10 ppm for male dogs and 20 ppm for female dogs based on the absence of proprioceptive placing. The NOAELs was 5.3 ppm for male dogs and 10.0 ppm for female dogs.

This six-week inhalation toxicity study in beagle dogs is **Acceptable/Nonguideline** and fulfills the intent of the study.

[It is noted that in a previous subchronic inhalation toxicity study (MRID 43386802), the systemic LOAEL (threshold) for a 7 weeks (34 exposures) was 5 ppm (0.021 mg/L), based on decreased responsiveness in females. The NOAEL (threshold) was <5 ppm.]

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METHYL BROMIDE/053201

Nonguideline

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Reregistration Branch 1, Health Effects Division (7509C)

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Signature: Paul ChinDate: 10/22/02Signature: Whang PhangDate: 10/22/02Signature: J. StewartDate: 10/22/2002

Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: Six-week Inhalation Toxicity Study - Dog (Nonguideline).**PC CODE:** 053201**TXR#:** 0050984**DP BARCODE:** D 284598**SUBMISSION NO.:** S619315**TEST MATERIAL (PURITY):** Methyl Bromide (100%)**SYNONYMS:** None reported

CITATION: Schaefer, G. J., (2002). A 6-week inhalation toxicity study of methyl bromide in dogs. WIL Research Laboratories, Inc., Ashland, OH. Laboratory Report No. WIL-440001. May 16, 2002. MRID 45722801. Unpublished.

SPONSOR: Alliance of the Methyl Bromide Industry

EXECUTIVE SUMMARY: In a six-week nonguideline inhalation toxicity study (MRID 45722801), four groups of beagle dogs consisting of 4 males and 4 females/group were administered methyl bromide (Lot No: 1010PK15A; purity: 100% a.i.) by whole body exposure at concentrations of 0, 5.3, 10, and 20 ppm. The exposures were for seven hours/day, five days/week for six weeks (total of 30 exposures). There were no compound related effects on mortality, clinical signs, body weight, food consumption, spleen weights, or gross or histological pathology. Functional observational battery and locomotor activity tests showed no abnormalities relative to controls with one exception. One male and one female at the 20 ppm dose and one male at the 10 ppm dose demonstrated an absence of proprioceptive placing, although no evidence of weakness in motor strength or other signs of neurotoxicity was found.

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This six-week inhalation toxicity study in beagle dogs is **Acceptable/Nonguideline** and fulfills the intent of the study.

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METHYL BROMIDE/053201

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

MATERIALS AND METHODS

A. MATERIALS:

1. **Test material:** Methyl bromide

Description: gas
Lot/Batch #: 1010PK15A
Purity: 100%
Compound Stability: Stable for the duration of the study
CAS # if TGA: 74-83-9
Structure: CH₃Br

2. **Positive controls:** Locomotor activity tests: Chlorpromazine hydrochloride (98%); amphetamine (>99%); dextroamphetamine sulfate (>99%).

3. **Test animals:**

Species: Dog
Strain: Beagle
Age/weight at study initiation: ~7 months/7.4 to 9.9 kg (males), 6.4 to 9.4 (females)
Source: Ridgman Farms, Mt. Horeb, WI
Housing: S.S. wire mesh suspended cages
Diet: 400 g daily, PMI Nutrition International, Inc. Certified Canine Diet 5007 *ad libitum*, except during exposure
Water: Tap water *ad libitum*, except during exposure
Environmental conditions:
Temperature: 19.5 to 22.8°C
Humidity: 29.8 - 74.9%
Air changes: Not reported
Photoperiod: 12 hrs light/dark
Acclimation period: 13 to 20 days

B. STUDY DESIGN:

1. **In life dates:** Start: December 19, 2001; End: February 8, 2002
2. **Animal assignment:** Dogs were randomly assigned to the test groups noted in Table 1.

TABLE 1: Study design					
Test group	Nominal Conc. (ppm)	Analytical Conc. (ppm)	MMAD (µm)	GSD*	Dogs/sex
Control	NA	0	NA	NA	4
Low (LCT)	8.9	5.3	NA	NA	4
Mid (MCT)	13	10.0	NA	NA	4
High (HCT)	27	20.0	NA	NA	4

NA = Not applicable

METHYL BROMIDE/053201

3. **Dose selection rationale:** A previously conducted pilot four-week inhalation toxicity study of methyl bromide in dogs was done to determine exposure levels for a proposed long-term inhalation study in dogs. In the pilot study, neurologic evaluations were limited to subjective observations by a veterinarian. The current study was conducted to resolve outstanding issues related to the equivocal observation of neurotoxicity (decreased responsiveness) in two female dogs exposed to 5 ppm for six weeks.
4. **Generation of the test atmosphere / chamber description:** The methyl bromide used in the current study was a liquefied gas. Test atmospheres were generated by metering the gas from the cylinder head space. To maintain the rate of vaporization, heat was applied to the outside of the cylinders using a thermostatically controlled heating blanket. Heat was also applied to the generation system components using heating tapes. An electric controller and a thermocouple positioned on the outside surface of the gas cylinders were used to provide a stable temperature.
5. **Test atmosphere concentration:** Nominal concentrations were calculated weekly by weighing the storage cylinders. Actual exposure concentrations were measured at 30-minute intervals by gas chromatography. Homogeneity of methyl bromide concentrations were verified in the development phases of the study. Time to chamber equilibrium was not reported.
6. **Statistics:** All analyses were conducted using two-tailed tests for minimum significance levels of 1% and 5% by comparing each treatment group to the control group by sex. Statistical analyses were not conducted if the number of animals was two or less. Body weights, body weight changes, food consumption, and Functional Observation Battery (FOB) data were subjected to a parametric one-way analysis of variance to determine intergroup differences. If the ANOVA revealed statistical significance ($p < 0.05$), Dunnett's test was used to compare the treatment groups to the control group. Discontinuous (ordinal of descriptive) Functional Observational Battery data were analyzed using Fisher's Exact Test to determine significant differences from the control group. Locomotor activity data were standardized (as percentages of study week -1 values) and analyzed using ANOVA. If statistical significance ($p < 0.05$) were found, Dunnett's test was used to compare the treatment groups to the control group. All statistical tests performed were appropriate.

C. **METHODS:**

1. **Observations:**

- 1a. **Cageside observations:** All animals were observed twice daily for toxicity and mortality.
- 1b. **Clinical examinations:** Clinical examinations were performed twice daily on exposure days, once at the midpoint of exposure (through the chamber) and again 0.5-1 hours following exposure. On non-exposure days, clinical observations were performed once in the morning. Detailed physical examinations were conducted on all animals weekly and on the day of necropsy.

2. **Body weight:** Individual body weights were measured weekly, beginning one week prior to test article administration. Mean body weights and mean body weight changes were calculated. Final body weights were recorded at necropsy.
3. **Food consumption:** Individual food consumption was recorded daily, beginning one week prior to test material administration and weekly thereafter. Food intake was calculated as g/animal/day for the same intervals. Food efficiency was not calculated.
4. **Neurobehavioral assessment:**
- 4a. **Functional observational battery (FOB):** FOB observations were recorded for all animals twice prior to the initiation of dosing (weeks -2 and -1), and approximately one hour following exposure during weeks 2, 4, and 6. Respiration and heart rates were also assessed. The time of testing was balanced across treatment groups. In order to accommodate this scheduling, one dog from each group was evaluated each evaluation day. The FOB was done in a sound-attenuated room equipped with a white noise generator set to operate at 70 ± 10 db. Home cage observations were done in the animal room. It was not reported whether the same technicians were used for each evaluation, or if they were blind to the treatment status of the animals. Positive control data from February, 2002 were reported on an unnamed chemical. All animals were observed for the following CHECKED (X) parameters.

HOME CAGE OBSERVATIONS		OPEN FIELD OBSERVATIONS	
X	General posture	X	Time to first step
X	General demeanor	X	Gait
X	Head posture	X	Behavior
X	Tremors/Convulsions		
X	Salivation		
X	Lacrimation		
X	Palpebral (eyelid) closure	X	Breaths per minute
X	Excreta examination	X	Heart rate and respiration pattern
SENSORY OBSERVATIONS			
X	Auditory response	X	Patellar reflex
X	Menace reaction	X	Perineal reflex
X	Pupillary reflex	X	Pinch reflex
X	Tracking	X	Cliff avoidance
X	Pupillary size	X	Proprioceptive positioning
X	Nystagmus (presence or absence)	X	Proprioceptive placing
X	Palpebral reflex	X	Posterior extensor thrust
X	Ocular position	X	Wheel barrowing
X	Righting reflex	X	Hemistanding/Hemiwalking
X	Triceps reflex	X	Jaw and tongue examination
		X	Gag reflex

- 4b. **Locomotor activity:** Observations were recorded for all animals twice before dosing (weeks -2 and -), and during weeks 2, 4, and 6 of the exposure schedule. The week-2 observations were conducted both to establish the dogs' normal behavior patterns and to allow the dogs to acclimate to the motor activity apparatus. Locomotor activity, recorded after the

completion of the FOB, was monitored using a MotorMonitor system (Hamilton-Kinder, Poway, California) in a room equipped with a white noise generation system set to operate at approximately 70 db. The testing of treatment groups was done according to a replicate sequence. Each animal was tested separately. Data were collected for 60 minutes at 15-minute recording intervals. Historical positive control data from April and October 2001 were reported on chlorpromazine hydrochloride, amphetamine, and dextroamphetamine sulfate.

5. **Ophthalmoscopic examination:** Not done.
6. **Hematology and clinical chemistry:** No hematology or clinical chemistry were done.
7. **Urinalysis:** No urinalysis was done.
8. **Sacrifice and pathology:** All animals were anesthetized with a barbiturate and euthanized by exsanguination. The dogs were perfused in situ with 4% paraformaldehyde and 1.5% glutaraldehyde to optimize the detection of neuropathological changes. All animals were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination (not all collected tissues were examined; emphasis was placed on the nervous system, while the lungs, heart, aorta, and lymph nodes were also examined). The (XX) organs, in addition, were weighed.

DIGESTIVE SYSTEM		CARDIOVASC./HEMAT		NEUROLOGIC	
X	Tongue	X	Aorta, thoracic	X	Brain
X	Esophagus	X	Heart	X	Peripheral nerve (bilateral)
X	Stomach	X	Bone marrow	X	Spinal cord
X	Duodenum	X	Lymph nodes	X	Pituitary
X	Jejunum	XX	Spleen	X	Eyes (optic nerve)
X	Ileum	X	Thymus	X	Trigeminal ganglia and nerves
X	Cecum		UROGENITAL		GLANDULAR
X	Colon	X	Kidneys	X	Adrenal
X	Rectum	X	Urinary bladder		Lacrimal
X	Liver	X	Testes	X	Parathyroid
X	Gall bladder	X	Epididymides	X	Salivary
X	Pancreas	X	Prostate	X	Mammary
	RESPIRATORY	X	Ovaries	X	Thyroid
X	Trachea	X	Uterus		OTHER
X	Lung	X	Vagina	X	Bone (sternum and/or femur)
X	Nose			X	Skeletal muscle
	Pharynx			X	Skin
X	Larynx			X	All gross lesions and masses

II RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** No treatment-related effects were noted during the study. One male dog in the 20 ppm group showed an impaired use of the left hind limb on one occasion. There were no correlating findings observed either microscopically or in the FOB, so this observation was not considered to be an adverse effect of test article exposure. No treatment-related effects of body temperature were noted for males or females at any dose level.
2. **Mortality:** All animals survived the 6-week exposure period.
- B. **BODY WEIGHT AND WEIGHT GAIN:** There were no treatment-related effects on body weight for males or females.
- C. **FOOD CONSUMPTION:** There were no treatment-related effects on food consumption

D. NEUROBEHAVIORAL RESULTS:

1. **Open Field and table top FOB observations:** In the 20 ppm group, an absence of proprioceptive placing was noted from one male during weeks 2, 4 and 6 and from one female during week 2. One male in the 10 ppm group also had an absence of proprioceptive placing during study weeks 2 and 4. In these animals, there was no evidence of weakness in motor strength. There were no other test article-related effects on mean open field or table-top observations. Differences from the control group were slight and were not statistically significant.

Home cage observations: There were no treatment-related effects on mean home cage observations.

2. **Motor activity:** There were no treatment-related effects on motor activity when either absolute or normalized (percent of study week -1 values) data were evaluated. Differences from the control group were slight and were not statistically significant (Table 2).

E. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** There were no absolute or relative to body weight changes in spleen weight, the only organ weighed.
2. **Gross pathology:** No treatment-related macroscopic results were identified at necropsy.
3. **Microscopic pathology:** There were no treatment-related effects in any of the organs or tissues examined.

TABLE 2. Motor activity (total activity counts/session) of dogs exposed to methyl bromide by inhalation				
Test week	Exposure concentration (ppm)			
	0	5	10	20
Males				
Pre-test	31,592 ± 21,170	21,482 ± 11,808	27,494 ± 3233	25,413 ± 7694
Week 2	41,510 ± 22,507	29,939 ± 9768	24,437 ± 17,364	31,299 ± 21,570
Week 4	34,564 ± 23,789	18,023 ± 9858	31,882 ± 18,978	43,534 ± 29,959
Week 6	39,144 ± 34,103	22,688 ± 13787	27,305 ± 18,432	44,329 ± 20,315
Females				
Pre-test	33,549 ± 12,487	34,323 ± 19,430	34,621 ± 23,394	32,194 ± 13,881
Week 2	28,134 ± 17,629	41,155 ± 12,345	39,668 ± 31,475	24,577 ± 14,421
Week 4	30,737 ± 24,213	41,941 ± 27,402	35,360 ± 20,815	37,441 ± 19,299
Week 6	38,922 ± 24,775	38,295 ± 28,363	40,642 ± 23,501	49,357 ± 7961

Data from Table 12, p. 113 - 116, MRID 45722801

Values represent mean ± s.d.

III. DISCUSSION AND CONCLUSIONS:

- A. INVESTIGATORS' CONCLUSIONS:** There were no test article-related deaths. Clinical observations, body weights, food consumption, body temperatures, FOB, and motor activity parameters were unaffected by test material exposure. No test material-related macroscopic or microscopic changes were observed at necropsy. Based on the results of this study, the no-observed-effect level (NOEL) for whole-body inhalation of methyl bromide was 20 ppm (HDT).
- B. REVIEWER COMMENTS:** A functional observational battery and locomotor activity tests predominantly showed no abnormalities relative to controls with one exception. One male and one female at the 20 ppm dose and one male at the 10 ppm dose demonstrated an absence of proprioceptive placing, although no confirmatory evidence of weakness in motor strength or other signs of neurotoxicity was found. The number of test animal was small, and the observation of the absence of proprioceptive placing was seen in one male (20 ppm) at several occasion and in one female. This finding indicated that neurotoxic effects of methyl bromide and it was consistent with toxicological profile of methyl bromide in producing neurological effects. The finding that of 1 male in 10 ppm group with the absence of proprioceptive placing could not be discounted. Therefore, the NOAEL for this study was 5.3 ppm, and the LOAEL was 10 ppm based on the absence of proprioceptive placing.

METHYL BROMIDE/053201Nonguideline

This six-week inhalation toxicity study in beagle dogs is **Acceptable/Nonguideline** and fulfills the intent of the study.

C. STUDY DEFICIENCIES: None.

DATA FOR ENTRY INTO ISIS

Subchronic Oral Study - nonguideline

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range ppm	Doses ppm	NOAEL ppm	LOAEL ppm	Target organ	Comments
053201	45722801	inhalation	dog	6 weeks	inhalation	whole body	5.3 - 20	0, 5.3, 10, 20	5.3 ♂ 10 ♀	10 ♂ 20 ♀	Nervous System	None

DATA EVALUATION RECORD

METHYL BROMIDE

STUDY TYPE: SIX-WEEK INHALATION TOXICITY STUDY - DOG

NONGUIDELINE

MRID 45722801

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
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AUG 30 2002

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Disclaimer

This review may have been altered subsequent to the contractors' signatures above.



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057445

Chemical: Methyl bromide

PC Code: 053201

HED File Code 13000 Tox Reviews

Memo Date: 10/22/2002

File ID: TX050984

Accession Number: 412-03-0019

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